THE BACTERIAL ORIGIN OF THE GUMS OF THE ARABIN GROUP.

By R. Greig Smith, D.Sc., Macleay Bacteriologist to the Society.

III.—The Acids produced during the Growth of *Bact. acaciæ* and *Bact. metarabinum* in Saccharose Media.

The two bacteria are grouped together because it became evident as the research proceeded that they produce identical acids. The preliminary tests, which were made with the view of obtaining a general idea of the nature of the acids so that a particular scheme might be adopted or devised, were made upon material which had been formed in chalk solutions of saccharose-potato extract. These solutions contained 5 % of chalk and 5 % of saccharose. Saccharose-potato extract had, in conjunction with agar and tannin, proved an excellent medium for the formation of gum, and on this account it was used pending the determination of the essential nutrients contained in it.

Flasks containing the media were infected with the bacteria and incubated for a month at 30°. At the end of this time, the cultures were evaporated, cooled, and treated with an excess of dilute sulphuric acid. There were thus obtained solutions of the bacterial acids and residues of calcium sulphate. The former were extracted with ether in the apparatus of Schoorl,* and the latter, after being dried in the air, were transferred to paper cartridges and extracted by ether diffusion in the same apparatus. After treatment with ether the respective extracts were

* Journ. Soc. Chem. Ind. xix., 567.

distilled to eliminate the ether and the residual fluids were examined.

The residue from the solution of the acid fluid was diluted with water when there separated out a small quantity of fat. This was not examined, partly on account of its small amount, and partly because a former experience had shown that the water insoluble fatty acids are found chiefly in the calcium sulphate residue. The filtered solution was distilled with steam until the distillate had but a faint acidity. The distillate, which had a fruity odour, was boiled with an excess of barium hydrate. What appeared to be common alcohol was detected as it rose in the aërial condenser attached to the flask in which the distillate was boiled. After boiling for two hours the solution was cooled, acidified with sulphuric acid and distilled. The distillate, which gave a very faint precipitate with mercuric chloride, was neutralised with baryta water and evaporated to dryness. The analysis of the residue, dried at 140°, gave the following figures:—

0·1444 grm. gave 0·1313 grm. $BaSO_4$ = 53·48 % Ba.
Barium acetate contains ... ... 53·73 % Ba.

During the analysis, the odour of acetic acid was given off upon the addition of the sulphuric acid to the barium salt.

The non-volatile acids were evaporated nearly to dryness, and allowed to crystallise overnight. Colourless prisms separated out. These could be sublimed and melted at 180°, thus indicating succinic acid.

After removing the crystals the mother liquor was diluted and a portion treated with calcium acetate. No precipitate formed at once, but on warming crystals slowly separated. When examined microscopically, after twenty-four hours, the crystals were seen to consist of tufts of needles with a few octahedra and lens-shaped forms. Both from the macroscopic and the microscopic observations the precipitate appeared to be calcium citrate with a trace of calcium oxalate. A solution of citric acid was treated with calcium acetate and used to

confirm the method of precipitation and microscopical examination.

The filtrate from the calcium citrate was treated with an excess of milk of lime and filtered. The residue obtained on evaporating the filtrate to dryness was extracted with hot 70 % alcohol and filtered. The filtrate, after standing overnight, had deposited mammillated crusts of what appeared to be calcium lactate. These were washed with cold alcohol and ether and finally dried at 100°. In the dry crystals the calcium was estimated :—

0·2740 grm. gave 0·1284 grm. $CaCO_3$ = 18·75 % Ca.
Calcium lactate contains ... ... 18·27 % Ca.

Another portion of the fixed acids, after separating the crystals of succinic acid, was boiled with an excess of baryta water, and neutralised with sulphuric acid. The barium sulphate was removed, and the clear filtrate treated with ammonia and three volumes of alcohol. The precipitate, when dried at 140°,*

* The barium salt was dried at 100° until it ceased to lose weight. On increasing the temperature to 140° a further loss of weight occurred. As the drying temperature would influence the analysis of the salt, a small quantity of succinic acid, which was one of the acids present in the solution, was neutralised with baryta water, and precipitated with alcohol, filtered, and dried at 100° and 140°. The following results were obtained :—

0·1 grm. succinic acid gave 0·2102 grm. barium succinate dried at 100°, and 0·206 2grm. dried at 140°.
The barium sulphate therefrom weighed 0·1874 grm.
Ba % at 100° = 52·43; at 140° = 53·44.
Ba in $BaC_4H_4O_4$ (theoretical) 54·16 %.
98·04 % of the succinic acid was recovered.

Citric acid was converted into the barium salt, and the following numbers were obtained :—

0·1 grm. crystallised citric acid gave 0·1840 grm. barium citrate at 100°, and 0·1740 grm. at 140°.
The barium sulphate therefrom weighed 0·1523 grm.
Ba % at 100° = 48·67; at 140° = 51·47.
Ba in $Ba_3(C_6H_5O_7)_2$ = 52·11 %.
98·53 % of the citric acid was recovered.

From these results it is evident that the barium salts must be dried at a temperature over 100°.

gave the following analysis:—

0·5962 grm. gave 0·5346 grm. $BaSO_4$ = 52·62 % Ba.
Barium citrate contains ... ... 52·11 % Ba.
Barium succinate contains ... ... 54·16 % Ba.

In view of my percentage figures being generally low, it would appear that the salt is a mixture of barium citrate and succinate.

The solution obtained after distilling the ethereal extract of the calcium sulphate residue was diluted with water when a brown insoluble fatty acid separated. This was collected, dried, transferred to a small filter, treated with ether, and the ether evaporated. The residual fatty acid, which was solid at the ordinary temperature, melted at 40-43°, from which it appeared to be lauric acid.

The mother liquor was boiled with an excess of baryta water, neutralised with sulphuric acid, filtered, and evaporated down to small volume. A salt separated out, and an analysis showed that it contained 51·94 % Ba, from which it appeared to be barium citrate.

The filtrate from the barium citrate was treated with an excess of sulphuric acid and distilled in a current of steam. No volatile acids passed over. The acid solution was extracted with ether, and the residual acids allowed to crystallise. Colourless prisms imbedded in lauric acid were obtained. The prisms were partly purified by drying them on filter paper at 100°; the fatty acid being absorbed by the paper. The crystals were sublimed and a determination of the melting point of the sublimate was made. The sublimate had the same microscopical appearance and melting point as sublimed succinic acid. It softened at 175°, and melted completely at 180°.

From these preliminary results it appears that the acids contained in cultures of the bacteria made in potato extract in the presence of chalk and saccharose consist of acetic, lauric, citric, lactic and succinic, with traces of formic and oxalic.

Since these acids were found in the potato extract cultures it is probable that in other and more definite media there

would be a smaller number, because the acids normally present in the extract are undoubtedly included. As the chief nitrogenous nutrient of the boiled potato extract is asparagine, I determined to employ it in media for the confirmatory tests. Accordingly, solutions containing saccharose, 50 grm.; asparagine, 3 grm.; potassium phosphate, 2 grm.; potassium chloride, 5 grm.; chalk, 10 grm. and tap-water 1000 c.c. were, after sterilisation, infected with the bacteria and allowed to stand in a cupboard at the ordinary room temperature for three weeks. In the culture of *Bact. acaciæ* the chalk granules, on shaking the flask, floated about loosely in the fluid, while with *Bact. metarabinum* the chalk and slime cohered together in one mass.

The culture of *Bact. acaciæ* was boiled under an inverted condenser with 4 grm. of barium hydroxide for two hours. The filtrate from the sediment was then distilled in a partial vacuum until one-third had passed over. The distillate was again distilled until one-third had distilled. The process was continued until about 10 c.c. of distillate were obtained. This had a smell of ammonia, which was removed by distilling with a few drops of phosphoric acid. Ultimately one c.c. of a fluid which showed the alcoholic tear-drops and boiled at 80° was obtained. From this a few drops of a liquid which boiled at 78° were obtained by distilling with anhydrous sodium carbonate. The liquid burned with a blue flame, and had the odour of ordinary ethyl alcohol. The residues obtained during the distillation gave the iodoform reaction with the characteristic microscopical appearance of iodoform.

The culture of *Bact. metarabinum* was heated on the steam bath with barium hydroxide as the viscous nature of the solution negatived any suggestion of boiling. Otherwise the process was a repetition of that to which the culture of *Bact. acaciæ* had been subjected, and as with *Bact. acaciæ* the culture of *Bact. metarabinum* yielded a few drops of ethyl alcohol.

The residual liquid from the first alcoholic distillation was evaporated down nearly to dryness, and when cold added to the barium-calcium carbonate sediment which had meanwhile

been treated with an excess of dilute sulphuric acid. After standing over-night the supernatant liquid was filtered and the residue washed with small quantities of water. Finally the residue was dried in the air and reserved for ether diffusion.

The filtrate, which had the odour of vinegar, was distilled in a current of steam, but as hydrochloric acid was found in the distillate the latter was returned to the original liquid and the whole was extracted by percolation with ether for twelve hours. Since hydrochloric acid had been found in the steam distillate of *Bact. acaciæ*, the distillation was not attempted with *Bact. metarabinum*. The ether was distilled off and the residual fluid reserved.

The barium-calcium sulphate residue, after drying in the air, was powdered and put into a filter paper cartridge and extracted by diffusion with ether. The ether was distilled off and water added to the residual fluid when an oil separated out. This was removed, dried, and the melting-point determined by the capillary tube method. In the tube the solidified fatty acid, which appeared microscopically as tufts of silky needles, rose at 43° and became clear at 45°. As the rising in the capillary tube is taken as the melting point of fats, the identity of this acid with lauric acid (m.p. 43·6°) may be assumed. The quantity was too small to warrant testing it by other means.

The mother liquor from the lauric acid was added to the solution of the acids obtained by the ethereal percolation of the acid solution, and the whole was distilled in a current of steam until a faintly acid distillate was obtained. Half of the distillate was neutralised with sodium hydrate, evaporated down to small bulk, and treated with silver nitrate. The white precipitate was quickly filtered and washed, then dried, first on porcelain, and finally over sulphuric acid *in vacuo*. When the salt ceased to lose weight an estimation of the silver was made.

0·2044 grm. gave 0·1354 grm. Ag = 66·25 % Ag.

Silver acetate contains ... ... 67·08 % Ag.

The chief volatile acid was therefore acetic. The filtrate from the silver acetate rapidly darkened, showing the presence of formic

acid. This was also shown by the decided formation of calomel on boiling the distillate with mercuric chloride.

The remaining solution of the volatile acids was evaporated to dryness after the addition of an excess of calcium carbonate. The dry residue was extracted with strong alcohol, and a portion of the solution tested with zinc nitrate; no precipitate of zinc valerate was formed. The remainder of the alcoholic solution was evaporated to dryness and the small residue was found to be insoluble in strong alcohol, and on the addition of dilute sulphuric acid, evolved the odour of acetic acid. Thus the only volatile acids that are formed by the bacteria are acetic and formic.

The non-volatile acids which had been set aside to crystallise produced colourless prisms that melted at 180°. They could be sublimed and a neutral solution formed a pale buff precipitate with ferric chloride. The crystals, therefore, were succinic acid.

Calcium acetate was added to the mother liquor and a slight precipitate was deposited in twenty-four hours. The precipitate consisted of microscopic octahedra of calcium oxalate.

The filtered solution was warmed, then placed in the water-bath, but no precipitate of calcium citrate could be obtained. Half of the solution was neutralised with milk of lime and returned to the remainder, but still no precipitation could be induced. Evidently citric acid is not a by-product of the bacteria, and in the preliminary experiments this acid must have been derived from the potato extract.

The solution was treated with an excess of milk of lime and filtered. The filtrate, after evaporation to dryness, was extracted with hot 70 % alcohol. The slight residue, insoluble in the alcohol, consisted of carbonate and succinate of calcium. Mammillated crystals of calcium lactate separated out from the alcohol on cooling, and the quantity showed that lactic acid* was the chief constituent of the non-volatile acids. The lactate was re-crystallised from alcohol (calcium succinate being found as an impurity) and an analysis made of the salt.

0·4965 grm. gave 0·2245 grm. $CaCO_3$ = 18·09 % Ca.
Calcium lactate contains ... ... 18·35 % Ca.

The purified salt from *Bact. metarabinum* was found to be dextro-rotatory, indicating that the acid contained laevolactic acid. The zinc salt of the acid produced by *Bact. acaciæ* was prepared and re-crystallised. It contained 13·1 % of water of crystallisation driven off at 140° C. As optically inactive lactate of zinc contains 3 molecules of water of crystallisation, equal to 18·18 %, and the active salt 2 molecules, equal to 12·9 %; it is evident that the acid consists chiefly of an active acid. The specific rotation of the hydrated zinc salt was found to be $[a_D] = +5·58°$, and of the acid to be $[a_D] = -3·69°$. According to Schardinger the specific rotation of laevolactic acid is $-4·3°$, and according to Purdie the pure hydrated zinc salt of laevolactic acid has a rotation $[a_D] = +6·81°$. The lactic acid formed by *Bact. acaciæ* therefore consists chiefly of laevolactic acid, and this undoubtedly also holds for *Bact. metarabinum.*

The mother liquor from the calcium lactate was evaporated almost to dryness and treated with strong alcohol. Crystals separated from the alcohol. These gave no odour of acetic acid on treatment with sulphuric acid. The analysis showed the following figures:—

0·0912 grm. gave 0·0542 grm. $CaCO_3 = 23·78$ % Ca.
Calcium aspartate contains ... ... 23·13 % Ca.

The precipitate was probably the calcium salt of aspartic acid, doubtless derived from the residual asparagine upon boiling the culture medium with barium hydroxide.

The method which was employed in the separation of the non-volatile acids is practically that of Schoorl; in the preliminary experiments it had been found to be most satisfactory. Malic acid could not be detected in the cultures.

The acids in the culture of *Bact. metarabinum* were identical with those obtained from *Bact. acaciæ*, with one exception. In place of lauric acid a mixture of a solid acid and another, fluid at the laboratory temperature, was obtained. This was peculiar because in the preliminary test the insoluble fatty acid was lauric and identical with that yielded by *Bact. acaciæ.* The quantity was, however, too small to separate the constituents.

During the fermentation of saccharose, carbon dioxide is evolved. This was proved by connecting flasks of baryta water with small cultures of the organisms in saccharose-potato extract. The usual precautions were adopted to seal the air inlet and trap the air outlet with a tube containing soda-lime. Upon drawing the air in the culture flask through the baryta water, barium carbonate was formed.

To gain some idea as to the relative quantities of volatile and non-volatile acids, a test was made with the acids obtained after extracting the sulphuric acid solution and the calcium sulphate residue of *Bact. metarabinum* with ether. These were added together, after filtering off the insoluble fatty acid, and distilled in a current of steam until the distillate had but a faint acidity. The non-volatile acids were maintained at 50 c.c. and the distillate measured 600 c.c. The volatile acids required 19 c.c., and the non-volatile acids required 59 c.c. of normal soda for neutralisation. The proportion is therefore, roughly, three parts of non-volatile to one of volatile acids.

In summarising these results it is seen that the acids formed by the action of *Bact. acaciæ* and *Bact. metarabinum* upon saccharose, with asparagine as a nitrogenous nutrient, consist of about three parts of non-volatile and one part of volatile acids. The former consist of laevolactic, chiefly, with a smaller quantity of succinic, of lauric and traces of oxalic. The volatile acids consist of acetic, chiefly, with a smaller quantity of formic and carbon dioxide. Ethyl alcohol is also formed during the fermentation.

IV.—The Gum-flux of the Vine.

The disease of the vine which is known by the name of "gummosis" or "mal nero," is characterised by the stems becoming stunted, the young branches do not develop normally, and the green leaves become deformed. Cross sections of the stem show the wood speckled with black in the earlier stages of the disease, and in the later stages the whole section is of a dark brown colour. The disease begins at the growing points,

generally at a wound, and spreads downwards. The microscopical examination of the wood shows the vessels, and especially the wood parenchyma, filled with a brown gum imbedded in which are myriads of bacteria.*

So far as I can learn, this disease is not found in Australia, but a gum-flux does occur. This is not a disease like gummosis, as the health of the plant is not appreciably affected. The gum exudes generally from the surfaces of the branches which have been cut by the pruning knife, and the vines which produce the gum are found in rather damp situations.

In response to my enquiries, Mr. Fred. Stoward, of Adelaide, S.A., forwarded several portions of vine stems with gum upon the pruned ends. The plants from which the portions were taken had been growing upon a low-lying, rather damp flat. The plants were not unhealthy, and the vigneron could not distinguish the vines which bore the gum from those in the same locality which yielded none. The sections of the branches had a normal healthy appearance. I also received a small quantity of dry gum which had been picked from the stems.

The gum consisted of small broken fragments, varying in colour from white to black. They were very dry and brittle, and broke with a glistening fracture. When covered with water the fragments swelled greatly, and the black colour was replaced by a brownish tinge. The gum softened and dissolved in water with extreme slowness. In this connection it must be borne in mind that the gum was collected in November and had been taken from wounds made by the pruning knife in the previous season. The gum had therefore been subjected to many months' rain, which would probably have washed away any soluble gum that might have been present originally. On boiling with 5 % sulphuric acid the gum acids were hydrolysed and were found to consist of arabinose and galactose, which showed that the gum was of the arabinan-galactan kind.

* Cent. für Bakt. 2te Abt. i. 300.

On cutting across a portion of the branch at the end of which gum was adhering, minute clear droplets issued from the cut ends of the large vessels of the wood. The droplets were, however, found to be sterile, and doubtless consisted of sap.

The transverse sections of the twigs and branches which had been sterilised on the outside by flaming, were inserted into nutrient glucose gelatine and incubated for from one to three hours at 30°. The infected media were subsequently poured into Petri-dishes and incubated at 22° for several days. Many colonies of bacteria developed upon the plates, and among them I identified *Bact. acaciæ* and *Bac. levaniformans*, both of which, as I have already shown, produce gum. The other bacteria could not be induced to form gum by the methods which had been successful in other cases, and it is probable that they were not gum-producing bacteria. I always purify the bacteria from the original colonies when they promise to be important, and in purifying one or two races of *Bact. acaciæ* I found *Bact. metarabinum*.

In the presence of *Bact. acaciæ*, *Bact. metarabinum* is not easy to separate. The deep colonies of both bacteria are very much alike, and the sub-surface colonies of *Bact. metarabinum* do not break through to the surface to form a slime-drop colony like *Bact. acaciæ*. It is only when the colony of *Bact. metarabinum* is actually on the surface that it can be recognised with certainty, and as there are comparatively few in original plate cultures, it is not surprising that *Bact. acaciæ* can be readily isolated and *Bact. metarabinum* can be easily ignored. In the original separation of *Bact. metarabinum* from *Acacia penninervis*, the bacterium had been picked out of the plates as being a sub-surface colony of *Bact. acaciæ*, and in the present instance its colonies had not been observed upon the original plates. The occurrence of *Bact. metarabinum* as an impurity in the original colonies of *Bact. acaciæ* is a point to be remembered when the organism is not found in the original plates.

With regard to the presence of *Bac. levaniformans* in the plant, it is probable that it is not responsible for the production of any

constituent of the gum. The gum levan which it produces from saccharose is readily hydrolysed in acid fluids, and would be hydrolysed by the acid juices of the plant as soon as it was formed. For this reason the presence of the organism may be looked upon as accidental. The secretion of invertase by this bacillus, however, must not be forgotten, and possibly the invertase may assist the other bacteria to form the gum.

The races of *Bact. acaciæ* and *Bact. metarabinum* were identical with those already described. Quantities of the gum were prepared by growing the bacteria upon saccharose-potato-tannin agar and subsequently obtaining the gum from the slime.* From the characters of the gum-acids as regards solubility and the formation of arabinose and galactose on hydrolysis, there was no doubt of the identity of the bacteria.

Summary.—The investigation showed that the gum-flux of the vine is caused by *Bact. acaciæ* and *Bact. metarabinum.*

V.—The Gum-flux of the Plum.

Among the Rosaceæ the plum frequently exudes gum from punctures and wounds on the stem and branches, and, like the gum from the other members of the family, plum gum is recognised as belonging to the arabin group.

I received specimens of wood and bark with adhering tears and globules of a pale straw to reddish-coloured gum from Mr. Cheel, who had obtained the specimens from the Crawford River district, about six miles from Bullahdelah. Unfortunately, the specimens as I received them were rather dry, a fortnight having elapsed since they had been removed from the tree. The gum masses, however, were large, and while the outer layers were

* The potato-extract used in the preparation of the agar was originally made by adding an equal volume of water to the juice of old potatoes. With new or early potatoes the juice may require to be much diluted. With certain potatoes I obtained the best results by adding 1 part of juice to 9 parts of water. This, however, will be discussed in a future paper upon the nutrition of the bacteria.

leathery the inner portions in contact with the bark were soft and probably contained living bacteria.

Accordingly, tubes of molten glucose-gelatine were infected with fragments of the soft gum, and some of these were poured into Petri-dishes at once, while others were poured after various periods of incubation at 30°. The colonies that developed from the plates were chiefly those of *Bact. acaciæ*. A few other bacteria were obtained, but as these could not be induced to produce gum upon saccharose-potato-agar, or the same with tannin, they were probably adventitious.

Most of the races of *Bact. acaciæ* were similar to the type which I have previously described, but another kind occurred which differed from the type in growing as a brownish-yellow mass on saccharose-potato-agar instead of the buff-yellow of the type.

The natural gum, from which the bacteria had been isolated, when treated with water partly swelled and partly dissolved. The portion which swelled showed rounded faces and corners; most of it dissolved in the course of a month, and doubtless it would all have dissolved in time. Upon hydrolysis with 5 % sulphuric acid the gum acids yielded a solution of reducing sugars which consisted of arabinose and galactose. These were identified by means of the osazones which were prepared and purified in the manner already described in the first of this series of papers.

In view of the slow solubility of the portion of the gum it appeared probable that *Bact. metarabinum* would occur among the bacteria, but although various methods were tried, and various media were employed, this organism could not be isolated. This failure to obtain the organism, however, does not necessarily imply that it had no part in the production of the natural gum. The separation is attended with difficulty on account of the insoluble nature of the gum which it produces. The slime masses of *Bact. metarabinum*, instead of dissolving and liberating the bacteria like *Bact. acaciæ*, remain intact, and thus a clump of bacteria grows as a single organism. Thus in plate culture *Bact. acaciæ* might so outnumber *Bact. metarabinum* as

to prevent the isolation of the latter. Again, the age of the specimens from which the bacteria were obtained might have much to do in bringing about the practical suppression of *Bact. metarabinum.*

One of the races of *Bact. acaciæ* had, however, differed from the normal type, and there was the possibility that this race might produce an insoluble gum, so in order to test this point a quantity of the slime was prepared. It was noted that the race was very vigorous, and produced a good quantity of slime. The pure gum acids, when taken from the alcoholic solution in which they had been precipitated, dissolved readily in water. Drying for several hours at 100° in the steam bath did not affect the ready solubility, from which it is evident that the bacterium was really a race of *Bact. acaciæ.*

The investigation showed that the gum-flux of the plum was due in part at least to the action of *Bact. acaciæ.*

VI.—The Gum-flux of the Cedar.

The gum-flux of the cedar has been already noted by Maiden,* who thus describes the gum—"It is a very pale yellow gum . . . swells largely in cold water, and in the course of 24 hours it nearly wholly dissolves . . . leaving a small percentage of metarabin."

A few twigs of the red cedar, *Cedrela australis*, F.v.M., bearing small amber-coloured tears of gum were forwarded to me by Mr. H. W. Potts, Principal of the Hawkesbury Agricultural College, and in the letter which accompanied the samples he said, "They were found on trees growing in Richmond. The gum appears to exude in all cases at points attacked by some grub, possibly that of the Red Cedar Moth, *Epicrosis.*"

Portions of the twigs were passed rapidly through the bunsen-flame to sterilise the outer surfaces, and were afterwards cut up with a sterile knife and introduced into tubes of molten glucose gelatine. Some of these tubes were poured into plates at once,

* Maiden, These Proceedings (2), iv. 1047.

others were incubated at 30° for 1, 2, 3, 4 and 5 hours before being poured into Petri-dishes. From all the plates colonies of bacteria were readily obtained, and most of these were *Bact. acaciæ*. The slime bacillus, *Bact. persicæ*, n.sp., first isolated from the peach, was also found, together with a few inert bacteria. The latter could not be induced to produce gum, and must therefore be considered as being adventitious saprophytes.

The gum-flux of the cedar is thus shown to be caused by *Bact. acaciæ*, the metarabin portion probably being contributed by the slime bacillus of the peach, *Baci. persicæ*, n.sp.

VII.—The Gum-flux of the Peach.

When the fruit of the peach-tree is affected with gum-flux, drops of an almost transparent, colourless and gelatinous gum exude from insect punctures or wounds upon the surface. Specimens of peaches diseased in this manner were received from Mr. H. W. Potts, Principal of the Hawkesbury Agricultural College. Some of the peaches contained the gum under considerable pressure, for upon cutting across the lower ends of the chambers containing the stones, large globules of gelatinous gum immediately protruded.

The microscopical examination of stained films of the gum showed the presence of immense numbers of small badly staining (and therefore probably dead) bacteria. In this respect, as well as in the microscopical appearance, the gum from the peach fruit was identical with gum from similarly affected almonds. In confirmation of the deduction drawn from the microscopical observations it was found that, although many portions of gum and gum-saturated tissue were infected into gelatine and other media, only a few colonies of bacteria were obtained. The few colonies that developed from about a dozen plates were those of *Bac. levaniformans* and another sporulating bacillus, *Bact. persicæ*, n.sp., which will be described in a future paper. A few yeasts were also obtained, but in view of the undoubted bacterial origin of the gum, as shown at any rate by the microscopical appearance

of stained gum films, they were considered as having nothing to do with the production of the gum.

The presence of dead bacteria in the gum found inside the fruit points to the gum being formed in the stem, and being forced into the rapidly growing fruit, where the bacteria are killed by the greater acidity of the fruit juices as compared with the stem fluids. If this be the case the bacteria which produce the gum should be found in the vessels of the twigs attached to the fruit. To test the matter fresh specimens of gummed peaches were obtained from Mr. Potts, and from these new cultures were made. The colonies that grew upon plates which had been infected with the twigs to which the fruits were attached consisted chiefly of *Bact. acaciæ*. The others consisted of the slime bacillus (*Bact. persicæ*, n.sp.), *Bac. levaniformans*, and a dematium-yeast which appeared capable of producing slime. From eight portions of gum and fruit pulp of the new specimens of fruit there were obtained an inert bacillus, an inert coccus, an inert sarcina, and many colonies of a small yeast and the dematium-yeast. In the twigs the yeasts were in the small minority, while in the fruit they predominated.

From this investigation it appears that the gum which exudes from punctures and wounds upon peaches, and is found saturating the soft tissues, has been formed in the stem and branches. The bacteria which produce the gum are chiefly *Bact. acaciæ*, but other bacteria also contribute, and the chief of these is the slime bacillus, *Bact. persicæ*, n.sp. *Bacillus levaniformans* is practically inert, for the gum levan that it produces would be immediately hydrolysed. This organism may, by virtue of its inverting action, play a part in assisting the other bacteria to produce gum, but this is doubtful.

The dematium-yeast grows as a tough skin upon saccharose-potato-agar, and possibly consists of cells cemented together or embedded in a slime matrix. It will form the subject of a future investigation. But even should it be proved to be capable of producing slime, its practical absence in film preparations of the fruit gum, and the overwhelming majority of bacterial remains,

is sufficient to show that the gum is a bacterial and not a yeast product. This is important because the gummosis (? gum-flux) of the plum has been ascribed to a similar dematium-yeast by Massee.*

The investigation showed that (1) the gum that exudes from peaches is formed in the stem and branches; (2) it has a bacterial origin, and (3) it is produced chiefly by *Bact. acaciæ*.

VIII.—The Gum-flux of the Almond.

The gum-flux of the almond showed† many points in common with the gum-flux of the peach. The fresh gum that exuded from punctures and cracks in the fruit was of the same colourless, almost transparent appearance and gelatinous consistency, and when examined microscopically the same badly staining short bacterial forms were observed. Moreover, when glucose-gelatine plates were prepared with media infected with portions of fresh gum and gum-saturated fruit-tissue, bacterial colonies were conspicuous by their absence; colonies of yeast-like organisms were obtained.

As in the case of the peach, many bacterial colonies and but few yeast colonies developed upon plates which were prepared with media infected with portions of twigs, the exterior of which had been sterilised by passage through a flame. The bacteria consisted chiefly of *Bact. acaciæ*. The other colonies, which were few in number, included *Bac. levaniformans*, the gum-levan organism, and the slime-forming bacillus which had been first isolated from the peach and which has been named *Bact. persicæ*, n.sp. The dematium-yeast also obtained from the peach was isolated, but as it constituted about 1 % of the colonies, it probably had little effect in determining the composition or nature of the gum.

The investigation showed that (1) the gum-flux of the almond is identical with the gum-flux of the peach; (2) the gum is a

* Massee, Kew Bulletin, 1899.

† The specimens of affected fruit were sent by Mr. Fred. Stoward, Adelaide.

bacterial product; and (3) the chief active organism is *Bact. acaciæ*.

IX.—The Gum-flux of an unknown Stock of the Japanese Date-plum.

A twig* of a seedling tree used as a stock for the Japanese date-plum, and apparently a species of Diospyros, showed small tears of an amber-coloured gum exuding from wounds upon the surface.

Two gum-producing bacteria were isolated by means of plate culture from the woody tissue. One of these was *Bac. levaniformans*, the other was *Bact. acaciæ*, which was undoubtedly responsible for the production of the exudation.

* The specimen was sent by Mr. H. W. Potts, Principal of the Hawkesbury Agricultural College.